REMARKS

Review and reconsideration of the Office Action dated July 25, 2005, is respectfully requested in view of the above amendments and the following remarks. Further, entry of present Amendment B is respectfully requested.

In view of the imminent approaching of the end of the Six Months' Statutory Period to respond to the Final Office Action of July 25, 2005, and in order to avoid abandonment of the application, a RCE is being filed herewith so Applicant's Amendment B may be timely entered and formally considered by the Examiner.

Claims 1, 10, 11, and 34 to 36 are under consideration. Claims 1 has been amended. Claims 2-9, 12-16, 32, and 33 have been cancelled. Claims 17 to 31 were previously withdrawn from further consideration pursuant to 37 CFR 1.142(b).

Support for the amendment to Claim 1 is found throughout the specification, i.e. paragraph [0058], [0059], [0061] etc. Examiner is reminded that support for Claim 34 can be found at paragraph [00117]; support for Claim 35 can be found at paragraph [00127]; and support for Claim 36 can be found at paragraph [00131]. Applicant has been careful not to add new matter to the Specification.

Office Action

Turning now to the Office Action in greater detail, the paragraphing of the Examiner is adopted.

Claim Rejections under 35 USC 112, First Paragraph

The Examiner has rejected claims 1, 10-11 and 32-36 for containing subject matter that the Examiner believes to be inadequately described in the specification as filed. The Examiner advanced reasons for this objection in the Action of January 10, 2005, and reiterates such objections once again. Below, the Applicant addresses the comments put forth by the Examiner on pages 3 to 11 of the instant Office Action, which are believed to also adequately address the issues raised in the Action of January 10, 2005.

Claim 1 has now been amended to recite: "**wherein the pathogen is HIV, HCV or Influenza virus**". In this way, the Examiner's objection has been met because adequate structure for HIV, HCV and Influenza virus would have been available to a person of skill in the art as of the priority date of the instant application. As of the priority date of October 9, 1998, adequate information regarding sequences of HIV, HCV and Influenza virus could be found in publicly accessible databases.

The sequences of HIV, HCV and Influenza virus are available through GenBank, and were available through this route as of the priority date of this application (October 9, 1998). GenBank is a comprehensive database that contains publicly available DNA sequences for more than 165,000 named organisms, obtained primarily through submissions from individual laboratories and batch submissions from large-scale sequencing projects. Examples of submissions for these viruses are provided below, along with accession numbers. These examples are merely a sampling of the publicly available sequences that provide structure of the viruses to which claim 1 is now limited.

Proteins, peptides and epitopes from HIV, HCV and Influenza virus were also known, and available through public databases at the time of the priority date of the instant application. It should be clear to the Examiner that because such sequences were available to the public as of the priority date, there would be no advantage to having included these sequences in the application at the time of filing. The great disadvantage of including such sequences in the application at the time of filing is that the application would be extremely lengthy, and would include an unwieldy sequence listing. It should now be clear to the Examiner, when taken together with the amendments and the information provided herein, that the publicly accessible databases are accepted by those of skill in the art as the only realistic repository of such sequence information. Paper copies or printed material bearing such sequence information is not searchable, and is not used by those of skill in the art.

Sample GenBank Sequences:

Accession: NC_002204: ***Influenza B virus RNA-1***, complete sequence (Total Bases Sequenced: 2368 bp) completed: Aug 2, **1993**. (with attributions to Kemdirim *et al*. Influenza B virus PB1 protein; nucleotide sequence of the genome RNA segment predicts a high degree of structural homology with the corresponding influenza A virus polymerase protein. Virology 152 (1), 126-135 (**1986**).

Accession: NC_002023: ***Influenza A virus segment 1***, complete sequence. With attribution to: Fields,S. and Winter,G.

Nucleotide sequences of influenza virus segments 1 and 3 reveal mosaic structure of a small viral RNA segment. Cell 28 (2), 303-313 (**1982**).

Accession: NC_001802: ***Human immunodeficiency virus 1***, complete genome. Number of Contigs: 72; Total Bases Sequenced: 9181 bp; submitted by direct submission on 12-NOV-**1997**, with attribution to Martoglio, *et al.* Signal peptide fragments of preprolactin and HIV-1 p-gp160 interact with calmodulin. EMBO J. 16 (22), 6636-6645 (**1997**).

Accession: NC_004102: ***Hepatitis C virus***, complete genome, submitted by Kolykhalov,A.A. and Rice,C.M. as a direct submission on 19-JUN-**1997**, with attribution to Kolykhalov, *et al.* Transmission of hepatitis C by intrahepatic inoculation with transcribed RNA, Science 277 (5325), 570-574 (**1997**).

The sequence of the HIV-1 genome has been known since approximately 1984. See, for example Hahn, *et al.* Molecular cloning and characterization of the HTLV-III virus associated with AIDS. Nature. 1984 Nov 8-14;312(5990):166-9 (PMID: 6095086); and Wain-Hobson, *et al.*, Nucleotide sequence of the AIDS virus, LAV. Cell. 1985 Jan;40(1):9-17(PMID: 2981635).

Los Alamos National Laboratory curates HIV databases containing data on HIV genetic sequences, as well as information on ***immunological epitopes***, drug resistance-associated mutations, and vaccine trials. As reported by personal communication with curator Dr. Brian T. Foley of the Los Alamos National Laboratory

(btf@lanl.gov), the website for these HIV databases (http://hiv-web.lanl.gov/content/index) was founded by Gerry Meyers in 1986, and has been available to the public since that date. The databases also give access to a large number of tools that can be used to analyze these data. This project is funded by the Division of AIDS of the National Institute of Allergy and Infectious Diseases(NIAID), a part of the National Institutes of Health(NIH).

Please find enclosed APPENDIX A, which is a screen shot of the website we referred to in the previous response of the Applicant dated May 13, 2005. This screen shot demonstrates that a number of sequence compendia did exist in 1998.

Publications identifying HIV epitopes are too numerous to list here, but a brief survey of the literature shows that epitopes are well known and published upon. See, for example Chandra, *et al.*, Epitope mapping of the low-molecular-mass subunits of reverse transcriptase in human immunodeficiency virus type 1 by monoclonal antibodies. Biomed Sci. **1990**; 1(5):507-12 (PMID: 1723005); Robert-Guroff, HIV-neutralizing antibodies: epitope identification and significance for future vaccine. Int Rev Immunol. **1990**;7(1):15-30 (PMID: 1722498); or Klasse, *et al.*, Differential IgG subclass responses to epitopes in transmembrane protein of HIV-1.Viral Immunol. **1990** Summer; 3(2):89-98. (PMID: 1694431).

The sequence of Hepatitis C Virus has been known since at least as early as 1997. Kolykhalov, *et al.*, published sequence information in the article entitled, *Transmission of hepatitis C by intrahepatic inoculation with transcribed RNA*, Science 277 (5325), 570-574 (1997). Further, this is but one example of the accessibility of HCV sequences to the public. Thus, it is

believed that the structure of HCV was accessible to the public as of the priority date of the instant application.

Publications identifying HCV epitopes are too numerous to list here, but a brief survey of the literature shows that epitopes are well known and published upon. See, for example Cerino, *et al.*, Identification of an immunodominant B cell epitope on the hepatitis C virus non-structural region defined by human monoclonal antibodies. J. Immunol. **1991** Oct 15; 147(8):2692-6 (PMID: 171757); Nasoff, *et al.*, Identification of an immunodominant epitope within the capsid protein of hepatitis C virus. Proc Natl Acad Sci U.S.A. **1991** Jun 15; 88(12):5462-6 (PMID: 1711232); or Brown, *et al.*, Improved diagnosis of chronic hepatitis C virus infection by detection of antibody to multiple epitopes: confirmation by antibody to synthetic oligopeptides. J. Med. Virol. **1992** Nov; 38(3):167-71 (PMID: 1283751).

The Influenza Genome Sequencing Project, funded by The National Institute of Allergy and Infectious Diseases (NIAID) curates an influenza database. The influenza database at http://www.flu.lanl.gov/ was started before the priority date of the instant application. Viral sequences are deposited in GenBank, and these data have been used to create the "flu" database and the Influenza Virus Resource. As reported through personal communication with Dr. Yiming Bao of National Center for Biotechnology Information (bao@ncbi.nlm.nih.gov), the earliest sequence for the full Influenza virus genome was known as of 1979 by Porter, *et al.*, Complete nucleotide sequence of an influenza virus haemagglutinin gene from cloned DNA. Nature 282, 471 - 477 (29 November **1979**). Prior to this, the earliest partial sequences were submitted in Both, *et al.*, Nucleotide sequence coding for the N-terminal region of the

matrix protein influenza virus. Eur. J. Biochem. **1979** May 15; 96(2):363-72.

Publications identifying Influenza virus epitopes are too numerous to list here, but a brief survey of the literature shows that epitopes are well known, and published upon. See, for example, Joassin, *et al.*, Monoclonal antibodies detect M-protein epitopes on the surface of influenza virions. Arch Virol. 1987;95(3-4):183-95(PMID: 2440414); Jones, *et al.*, Degeneracy of T cell receptor recognition of an influenza virus hemagglutinin epitope restricted by HLA-DQ and -DR class II molecules. Eur. J. Immunol. 1994 May;24(5):1137-42 (PMID: 7514130); or Daly, *et al.*, Immunodominance of major histocompatibility complex class I-restricted influenza virus epitopes can be influenced by the T-cell receptor repertoire. J. Virol. 1995 Dec; 69(12): 7416-22. (PMID: 7494246).

The Applicant believes that the specification indeed provides adequate description of a process for preparing an immunogenic peptide mixture, starting with sequences of HIV, HCV or Influenza virus, as would have been available to those of skill in the art. A number of such mixtures are described in the Examples. Specifically, mixtures directed to HIV, HCV and Influenza virus are disclosed, and the preparation of such mixtures is based directly on the method provided. Although the Applicant believes that the method is widely applicable to a number of pathogens, in the interests of expediting prosecution, the process has been limited in amended claim 1 to HIV, HCV and Influenza virus.

The Applicant does not wish to limit the process to recite particular peptides from an exemplary mixture, since a person of skill in the art can easily access sequences for HIV, HCV and

Influenza virus. The Applicant believes adequate structural formula is now provided by limiting the claims to the recited pathogens. The Applicant advises that those skilled in the art would understand how and where to obtain information regarding immunogenic epitope sequences for HIV, influenza, and HCV.

The Examiner states that synthesis of a peptide requires a structure to enable synthesis, such as for chain lengthening. This is precisely the process that the instant claim set is directed to. The process outlines the steps needed to *arrive at* the amino acids that are to be added in a chain lengthening procedure, starting with the very first amino acid.

It is believed that with amendments now in place in claim 1 that the invention is fully supported. By providing structure through reciting HIV, HCV and Influenza Virus, each having a known structure, it is believed that the objection raised and supported by the citation of University of California v. Eli Lilly (43 USPQ 2d 1398, 1405 (1997) has been traversed. Claim 1 now recites "obtaining from a database. . .," which should provide clear direction to a person of skill in the art.

In order to more fully explain to the Examiner how those skilled in the art can identify variable vs. conserved epitopes, the Applicant provides a sample of sequences in APPENDIX B, showing actual HIV-1 sequence alignments from an HIV-1 sequence compendium from 1998. The conserved region is shown in the reference sequences, an in the sequences listed below the reference sequence, it is clear that there are very few differences among isolates obtained from infected individuals. However, looking at the variable region, there are a number of residues that differ between the reference sequence and the sequences from the isolates. When interpreting the sequence

alignments shown in APPENDIX B, please refer to the legend appearing above the alignments, which indicates that for example, "-" means an amino acid is identical to that of the reference strain.

Other points the Applicant wishes to emphasize in APPENDIX B:

A. Brackets are used to identify readily observed conserved vs. variable regions. It is not difficult to identify a variable region. Importantly, there are a few random amino acid differences even in the conserved regions, but these do not approach the lower limit specified claim 1.

B. The five hyper-variable regions of the gp120 protein were defined in the published sequence compendia, making very clear where a variable region is located. It is these very regions to which the invention is applied.

C. A box is used to define the location of sequences used in preparing a formulation according to the invention (labeled as "Variosite formulation #3"). The formulation itself, with the variable positions shown, is listed below the box. Thus, it should be readily apparent to the Examiner by briefly reviewing the residue(s) at each position, why the formulation has a single, or a multiple amino acid(s) present.

It is emphasized that APPENDIX B illustrates just a fraction of the hundreds of sequences assessed, and so APPENDIX B is illustrative only, and does not show all of the sequences used to arrive at the selected residues and frequencies (thus, one cannot calculate the frequencies precisely based on only the sequences shown in APPENDIX B). It is hoped that the

illustration provided in APPENDIX B is helpful to the Examiner's understanding of the process of the invention.

The Applicant has now met the requirements of the Examiner and traversed the objections raised under 35 USC 112, (first paragraph), specifically: the claims have been amended to now provide structures from which the variable regions may be selected. The process claims of the invention are now restricted to the specific pathogens as indicated, for which the structures (sequences) are known, and for which there is specific disclosure support within the application as filed.

Withdrawal of the rejection is respectfully requested.

Claim Rejections under 35 USC 112, Second Paragraph

In response to the objection raised to new claim 33, this claim has been cancelled.

Claim Rejections under 35 USC 103

The Examiner continued to reject Claims 1 and 10-11 as amended under 35 U.S.C. 103 (a) as being obvious over Anderson, et al. The Examiner further, rejected claims 32-36, under 35 U.S.C. 103 (a) as being obvious over Anderson, et al.

In response Claims 32 and 33 have been cancelled.

Further, the Applicant believes that the teachings of Anderson, *et al.* do not render obvious claim 1 as amended. There is no implication in Anderson, *et al.* that the number of different peptides in the mixture be limited. The desire to limit in any way the complexity of the mixture formed according to the process was not discussed in Anderson, *et al.* or in any other prior art document. The process of the instant invention

reduces complexity in the resulting mixture in a way that was never taught or suggested by Anderson, *et al.*

The Anderson, *et al.* paper did not suggest a reduction in complexity, and no intervening publication teaches or suggests any advantages of reducing complexity. Indeed, the Anderson, *et al.* paper teaches away from the concept of limiting complexity, and instead emphasizes enhanced complexity, by formulating a mixture with every possible peptide combination with a large number of variable residues represented in thousands of possible peptides. In the years since the Anderson *et al* publication, prior to the filing of the instant application, it is clear that no group (including the inventor's own lab publications) considered that a functional mixture could be prepared on the principle of reduced complexity. Certainly a complexity of 64 peptides or fewer was not conceived of, as now claimed in the instant claim set.

The process described by Anderson, *et al.* would not give rise to a vaccine that has any potential for commercial use. One reason the mixture of peptides described by Anderson, *et al.* would not be commercially feasible is that there are regulatory requirements to disclose the contents of a mixture, and to achieve batch to batch consistency. Because the complexity of the Anderson, *et al.* mixture would result in many thousands of different peptides together in a mixture, the mixture would be onerous to characterize, in an effort to meet regulatory approval requirements. Additionally, the larger the number of peptides, the greater is the likelihood of batch-to-batch inconsistencies when many thousands of different peptides are combined, compared to when up to 64 peptides are found together in a mixture. In this way, the mixture of the instant

invention, in which variability *(complexity)* is limited, has less likelihood of inconsistency due to the reduced number of mixture constituents. In the previous response, the Applicant did not wish to imply that the instant invention has already achieved commercial success, but rather attempted to indicate that, due to reduced complexity in the mixture, the instant invention results in a mixture that has a reduced likelihood of inconsistencies that would hinder regulatory approval, which is prerequisite to commercial viability. It is hoped that this point has now been clarified.

At the time of the in Anderson, *et al.* publication, it was not recognized that a process that *limits* variability would actually have an effective anti-viral outcome, which has now been shown to be the case with the Applicant's process.

The mixture prepared according to Anderson, *et al.* would lead to over 32,000 variants (see table 1 on page 737). Characterization of the mixture alone would be an onerous task. On page 736 (final sentence) Anderson, *et al.* state: "Thus, in a single synthesis, a HEC consisting of a mixture of peptides representing ***all*** the observed *in vivo* variants of an epitope was produced." [Emphasis added]. This passage indicates that there was no recognition that limiting the complexity of the mixture was desirable or posed any advantage to the mixture whatsoever.

Claim 1 as now amended clearly limits the number of peptides in a mixture to between 2 and 64. This means that a peptide mixture can have at maximum 6 variable residues, when only 2 options per variable residue are identified, leading to a total number of peptides of 2^6 or 64. The process of claim 1 has a number of other distinctions not taught by Anderson, *et al.* In particular, the rules by which amino acids representing

variable residues are selected are clearly presented, in terms of a threshold frequency which must be identified, a rounding step that is not employed in the Anderson, *et al.* paper.

In the discussion section of the Anderson, *et al.* paper (page 739, second paragraph), it is stated that an "HEC, representing ***tens of thousands*** of slightly different variations of an epitope, should ensure that peptides will exist, which can overcome the problems described above" [Emphasis added]. This passage clearly illustrates that Anderson, *et al.* believed at the time that each possible required peptide must be in the mixture, in order to address the problem.

The rationale put forth in the previous response to illustrate that the Examiner failed to meet the burden of factual support for *prima facie* obviousness is not reiterated here, for the sake of brevity, but all arguments are again incorporated by way of reference to the response of May 13, 2005.

On page 13 (lines 1 to 4), the Examiner has replied that the ". . .Applicant's peptides would amount to a similar, if not the same 32,000 peptides. . ." The Applicant emphasizes that it is the process itself that is being claimed. The process has never been used, and would not be obvious to a person skilled in the art, regardless of whether one or more peptide formed in the process was previously formed or know.

On page 13, lines 4 to 6 the Examiner asks how "only two peptide mixtures [can] be obtained from several epitope variations of a given pathogen, especially when the structures of a pathogen is not even known." In reply, claim 1 has now been amended to recite HIV, HCV and Influenza virus as the pathogen, and these structures are known. If the Examiner is

wondering how the process would work if there was only one variable residue identified, and thus, only two options at the variable residue, this would be a possibility if, for example, the variable region (such as the one shown in APPENDIX B) contained a stretch of a sequence having a variety of variable residues, but only one meeting the criteria (as set out in claim 1) of having a frequency greater than a threshold frequency of about 12 % (which is then rounded up to the nearest 25%). In this way it is possible to have a peptide mixture with only two peptides.

On page 13 (lines 7 to 9) the Examiner alleges that the percentages in the Anderson, *et al.* publication of 20%, 30% and 80% are not adequately different from the "nearest 25%" indicated in claim 1. The Applicant respectfully disagrees. In the instant invention, an amino acid to be added at a variable residue can only be added at one of three levels: 25%, 50%, and 75% (corresponding to 1/4, 1/2, or 3/4). In the prior art teachings of Anderson, *et al.*, an amino acid can be added at one of any variety of levels, which include 20%, 30%, 80% (corresponding to 1/5, 3/10 and 4/5). If a mixture contains 3 variable residues (thus, 8 different peptides), the method according to the instant application contains the 8 peptides in predictable proportions, ranging from 1/64 (one out of every 64 peptides would contain all three amino acids listed at the 25% level for the variable residues) to 27/64 (27 out of every 64 peptides would contain all three amino acids listed at the 75% level for the variable residues). This predictability cannot be projected onto mixtures prepared with non-standardized ratios at the variable residues. An almost limitless number of proportions of the resulting 64 peptides can be envisioned if

the ratios of variable residue amino acids are not standardized. As a comparative example, if the 20% level is selected for all three variable residues, the proportion of resulting peptides containing all three of the least frequently occurring residues would be 1/125, whereas the proportion of resulting peptides containing all three of the most frequently occurring residues (at 80%) would be 64/125. As a further comparative example, if 10%, 13%, and 15% levels were selected for all three variable residues, the resulting proportions of peptide containing all three of the least frequently occurring residues would be about 1/513, and the proportion of the peptide containing all three of the most frequently occurring residues would be about 341/513. Without any limitations or restrictions on the frequencies with which variable residues can be represented, an almost limitless number of proportions of resulting peptides can be envisioned, some of which would be present in very minor fractions. According to the example given (with three variable residues), the minimum proportion of any particular peptide present in the mixture would be 1/64.

The predictability of occurrence of any particular sequence within the peptide mixture is an important aspect of the limited variability that is an important feature of the instant invention, and appears to be an aspect of the invention that the Examiner failed to appreciate previously as a difference between using any variety of proportions of variable residues. The Applicant believes that this systemization has produced the beneficial result of limiting proportions within the mixture, and has the unexpected result that the mixture retains the desired effect, regardless of whether the proportions of amino acids present at the variable residues adheres exactly to the

ratio determined in the alignment. In the prior art, others, upon finding a 20%:80% ratio would not have changed the ratio to a 25%:75%. In fact Anderson, *et al.* teaches away from modifying the ratio in this way, since the ratio used is the ratio found in the alignment. There is no motivation found in Anderson, *et al.* to modify this ratio to reduce variability in the proportions of the resulting peptides in a mixture. Nor has any document been provided by the Examiner to combine with Anderson, *et al.* that suggests that such modified ratios be attempted.

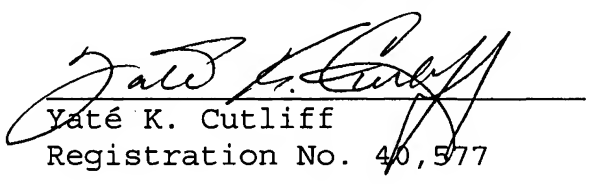
On page 14 of the Action (lines 13 - 16), the Examiner states that Anderson teaches or suggests all of the claimed limitations. The Applicant believes that the steps in the process that employ the nearest 25% for amino acids a variable residue are contrary to the teachings of Anderson, *et al.*, in which the ratios found in the alignment are used directly, and without modification toward a set ratio.

In view of the present amendment and foregoing remarks, reconsideration of the application is respectfully requested.

Withdrawal of the rejection is respectfully requested.

Favorable consideration and early issuance of the Notice of Allowance are respectfully requested. Should further issues remain prior to allowance, the Examiner is respectfully requested to contact the undersigned at the indicated telephone number.

Respectfully submitted,



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Date: **October 6, 2005**

CERTIFICATE OF MAILING AND AUTHORIZATION TO CHARGE

I hereby certify that a copy of the foregoing AMENDMENT B and Request for Continued Examination for U.S. Application No. 10/072,084 filed February 8, 2002, was deposited in first class U.S. mail, postage prepaid, addressed: Mail Stop: Amendment, Commissioner for Patents, P.O. Box 1450, Alexandria VA 22313-1450, on **October 6, 2005**.

The Commissioner is hereby authorized to charge any additional fees, which may be required at any time during the prosecution of this application without specific authorization, or credit any overpayment, to Deposit Account No. 16-0877.

Yaté K. Cutliff

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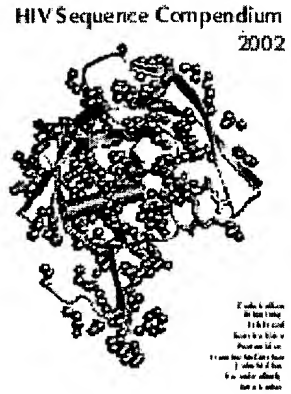

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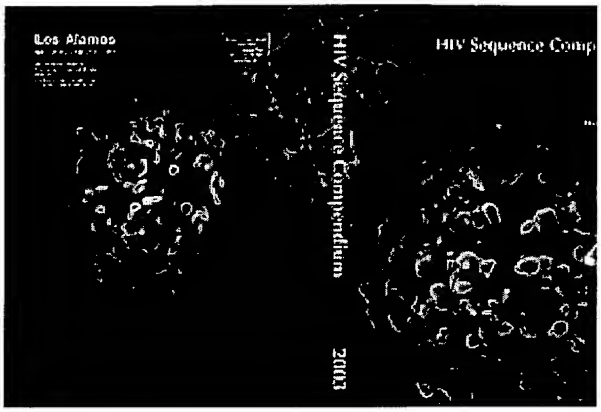

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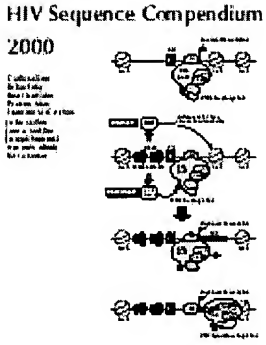

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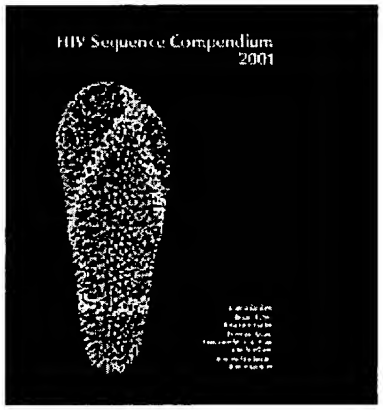

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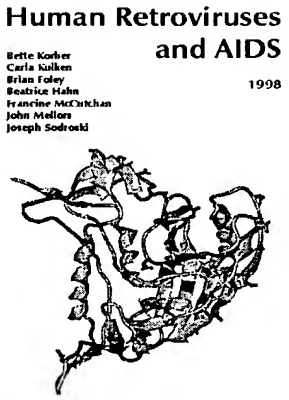

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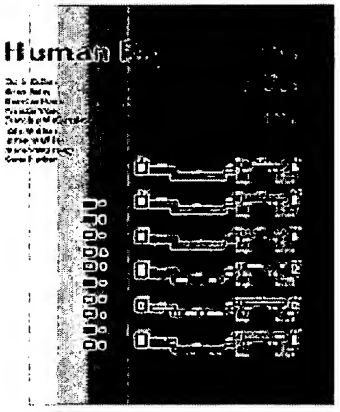

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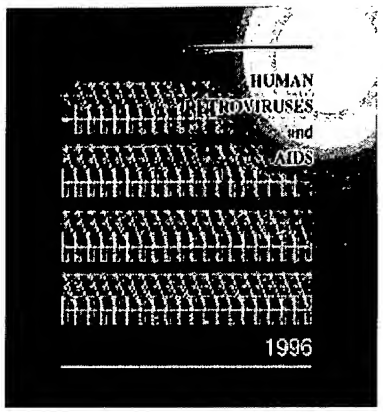

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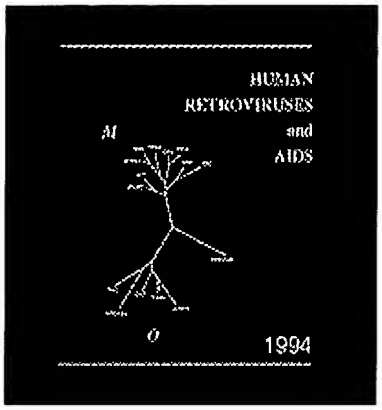

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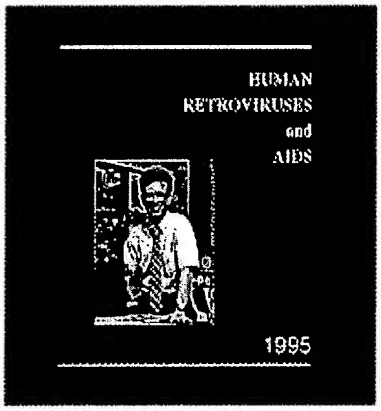

Last modified: Fri Dec 17 1:

Questions or comments? Contact us at seq-info@t10.lanl.gov

APPENDIX A PAGE 2 OF 2

Note: "-" indicates that an amino acid is conserved relative to the reference sequence, while "." are introduced to achieve optimal sequence alignments; the locations of the 5 hypervariable regions of HIV-1 gp120 (V1-V5) were indicated in the published sequence compendium. By way of comparison, conserved and variable regions have been indicated below.